Supporting information

Preparation of cellular membrane-mimicking glycopolymer interfaces by the solvent-assisted method on QCM-D sensor chips and their molecular recognition

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1. Materials and Methods

Butyl acrylate (BMA, 99%), lithium bromide (LiBr, 99%) and *N*,*N*-dimethylacrylamide (DMA, 99%) were purchased from Tokyo Chemical Industry (Tokyo, Japan). 2,2'-Azobis(isobutyronitrile) (AIBN), 1,4-dioxane, phosphate-buffered saline (PBS, 10 mM of phosphate) and ethanol were purchased from Fujifilm Wako Pure Chemical Industries (Tokyo, Japan). Bovine serum albumin (BSA) was purchased from Sigma Aldrich (St. Louis, USA). Peanut agglutinin (PNA) was purchased from Vector laboratories. Quartz crystal microbalance with dissipation (QCM-D) sensor chip with glass coating (QSX 303 SiO_2) was purchased from Q-sense. Methyl 2-(butylthiocarbono-thioylthio)propanoate (MCEBTTC)[1] and mannose acrylamide[2] was prepared according to previous papers. Commercial monomers and 1,4-dioxane including the radical inhibitor were purified by passing through an alumina column prior to use. For preparation of PBS(+), $CaCl_2$ (1.8 mM) and $MgCl_2$ (0.5 mM) were added to PBS solution.

  Proton and carbon nuclear resonance (^{1}H NMR) spectra were recorded on a JEOL-ECP400 spectrometer (JEOL, Tokyo, Japan) using chloroform-*d* or D_2O as a solvent. Size exclusion chromatography (SEC) analysis was performed on a HLC-8320 GPC Eco-SEC equipped with a TSKgel Super AW guard column and TSKgel Super AW (4000 and 2500) columns (TOSOH, Tokyo, Japan). The SEC analyses were performed at a flow rate of 0.5 mL/min by injecting 20 μL of a polymer solution (2 g/L) in DMF with 10 mM LiBr. All the samples for SEC were previously filtered through a 0.45 μm filter. The SEC systems were calibrated using a poly(methyl methacrylate) standard. Dynamic light scattering (DLS) measurements were performed on a ZETASIZER NANO-ZS (Malvern, UK) by using a 1 mL disposable cell of a polymer solution (0.1 g/L) in the PBS buffer solution. QCM-D measurements were performed on a Q-sense Explorer instrument (Biolin Scientific, Sweden). Changes in the resonance frequency (ΔF) and energy dissipation (ΔD) of the oscillating sensor chip (QSX 303 SiO_2) as a function of time were simultaneously recorded at multiple odd overtones (3rd, 5th, 7th, 9th, and 11th). All data displayed represent recordings at the 7th overtone. The temperature of the flow cell was fixed at 25 °C. Atomic force microscopy (AFM) was performed with a DimensionIcon (Bruker). AC mode topography and QI images were obtained in air using cantilevers (SCANASYST-AIR, Bruker) with a nominal spring constant of 10−130 Nm^{-1} and a resonance frequency of 70 kHz. Images were analyzed with the data analysis software NanoScope Analysis (version 1.8).

2. Synthesis of the amphiphilic copolymers
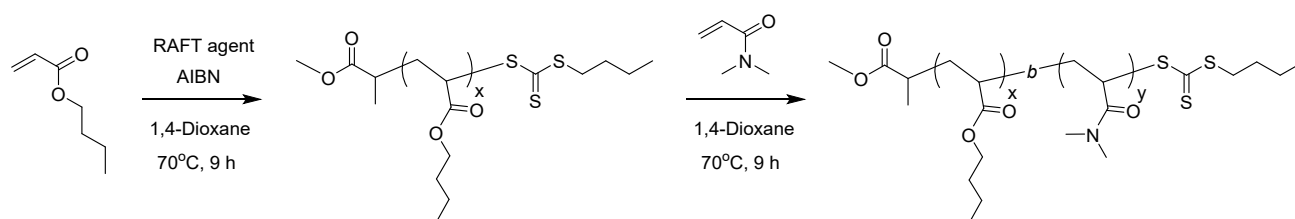
BA, MCEBTTC (RAFT agent) and initiator (AIBN) were dissolved in 1,4-dioxane. The monomer concentration and the ratio of reactants are shown in Table S1. The ratio of [RAFT]/[initiator] was set at 1:0.2. The solution was prepared in a glass tube and degassed by freeze−thaw cycles (three times). The glass tube was sealed and put in an oil bath. The reaction proceeded at 70 °C for 9 h. The reaction was stopped by exposing the solution to air. The monomer conversion was determined by ^{1}H NMR (CDCl$_3$). The polymer solutions were diluted with THF (2 mL) and reprecipitated in a mixture of water (15 mL) and MeOH (10 mL). The precipitated polymers were collected with CH$_2$Cl$_2$ and washed by brine to remove water. The organic phase was dried *in vacuo*, and polyBA$_x$ was obtained as yellow liquid. For diblock copolymers, DMA, PolyBA$_x$ (macro-RAFT agent) and AIBN were dissolved in 1,4-dioxane. The reaction proceeded in the same method as PolyBA$_x$. The polymer solutions were diluted with MeOH (2 mL), followed by dialysis against Milli-Q water (MWCO = 3.5k). The diblock copolymers were obtained by freeze-drying.

Table S1. Results of RAFT polymerization for polybutylacrylate.

First block	BA g (mmol)	MCEBTTC mg (μmol)	AIBN mg (μmol)	1,4-Dioxane (mL)	[Monomer] (mol/L)	[M]/[RAFT]/[Ini]	Time (h)	Conv. (%)	M_n (g/mol)	M_w (g/mol)	M_w/M_n	DP from NMR
PolyBA$_{50}$	1 (7.8)	39 (156)	1.3 (7.8)	2	3.9	50: 1: 0.05	15	98	6,900	8,300	1.21	58
PolyBA$_{100}$	1 (7.8)	20 (78)	0.64 (3.9)	2	3.9	100: 1: 0.05	15	96	14,000	16,400	1.18	110
PolyBA$_{200}$	1 (7.8)	10 (39)	0.32 (2.0)	2	3.9	200: 1: 0.05	15	96	29,600	36,200	1.11	196

Table S2. Results of RAFT polymerization for the amphiphilic block copolymers.

Diblock copolymers	PolyBA mg (μmol)	DMA mg (μmol)	AIBN mg (μmol)	[Monomer] (mol/L)	[M]/[Macro RAFT]/[Ini]	Time (h)	Conv. (%)	M_n (g/mol)	M_w (g/mol)	M_w/M_n	DP of DMA from NMR
Poly(BA$_{58}$-*b*-DMA$_{20}$)	100 (13.5)	29 (290)	0.12 (0.73)	1	20: 1: 0.05	12	>99	8,300	11,300	1.36	24
Poly(BA$_{58}$-*b*-DMA$_{50}$)	100 (13.5)	72 (725)	0.12 (0.73)	2.4	50: 1: 0.05	12	>99	12,200	14,800	1.21	64
Poly(BA$_{110}$-*b*-DMA$_{50}$)	100 (7.1)	35.5 (358)	0.06 (0.36)	1.2	50: 1: 0.05	12	>99	16,100	21,400	1.33	55
Poly(BA$_{110}$-*b*-DMA$_{100}$)	100 (7.1)	71 (716)	0.06 (0.36)	2.4	100: 1: 0.05	12	>99	22,100	26,400	1.20	110
Poly(BA$_{196}$-*b*-DMA$_{100}$)	100 (3.9)	39 (390)	0.03 (0.2)	1.3	100: 1: 0.05	12	>99	31,800	42,400	1.34	121
Poly(BA$_{196}$-*b*-DMA$_{200}$)	100 (3.9)	77.3 (780)	0.03 (0.2)	2.6	200: 1: 0.05	12	>99	44,100	57,300	1.30	233
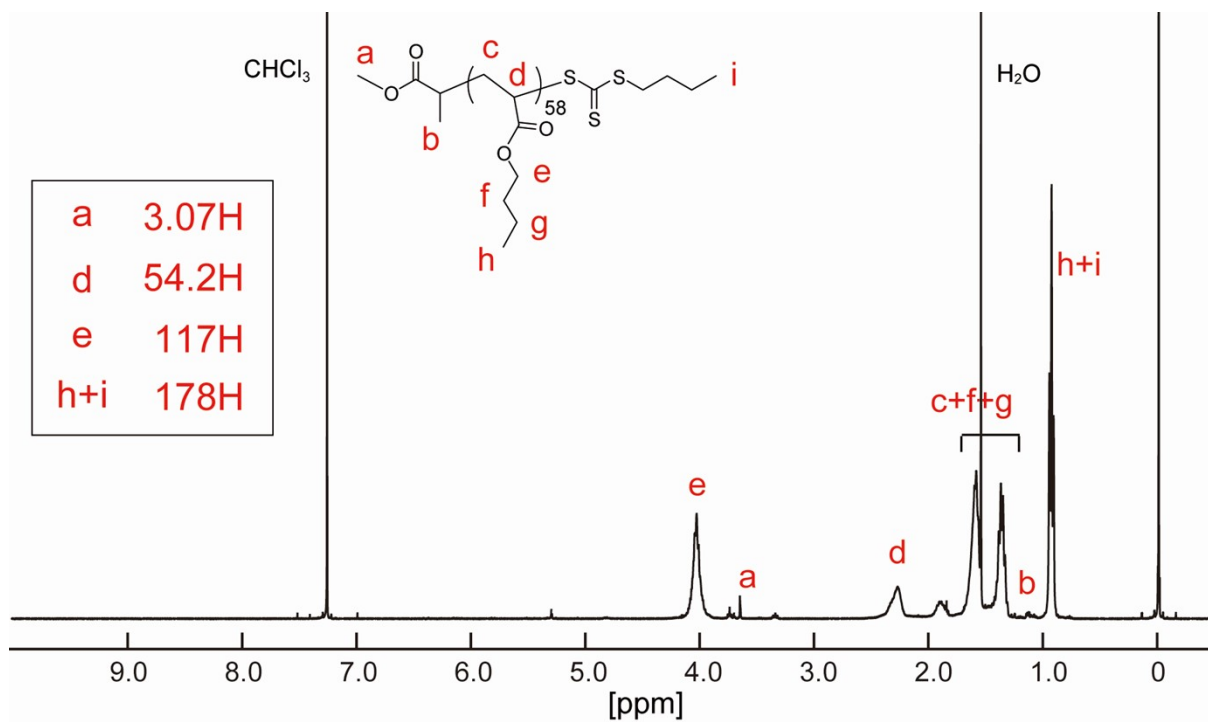
Figure S1. ^{1}H NMR spectrum of polyBA$_{58}$ (400 MHz, CDCl$_3$).
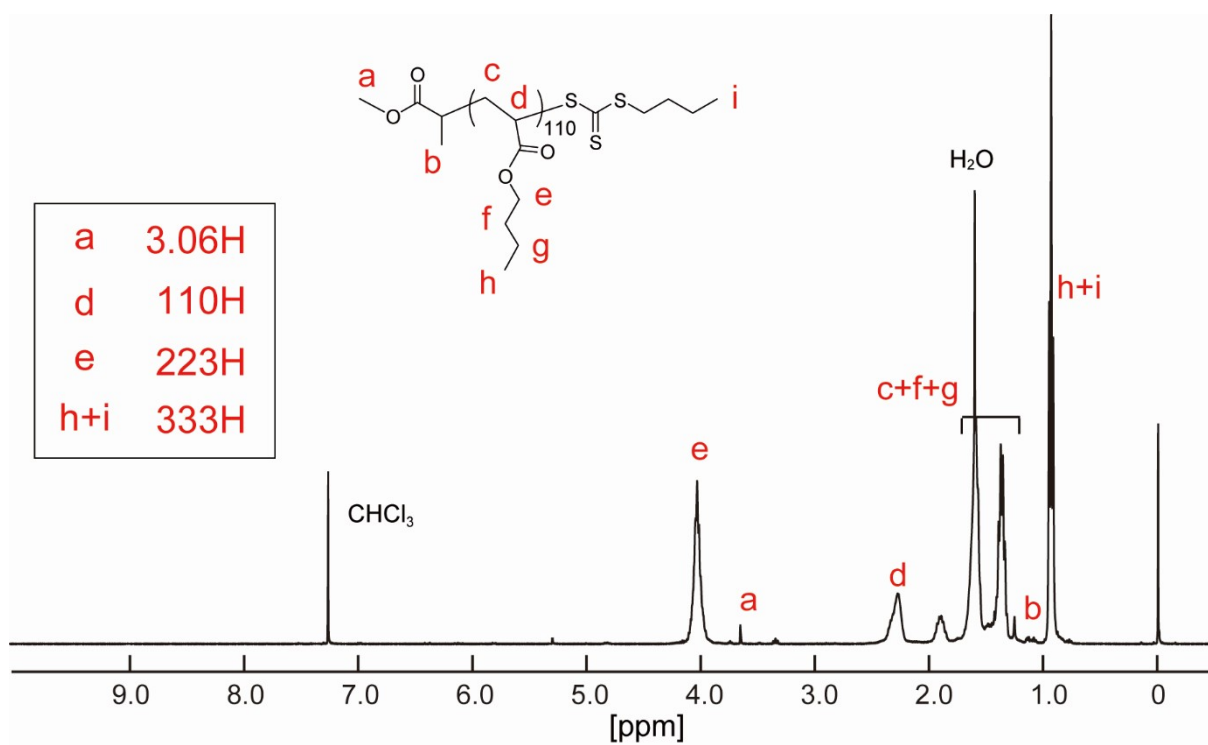
Figure S2. ^{1}H NMR spectrum of polyBA$_{110}$ (400 MHz, CDCl$_3$).

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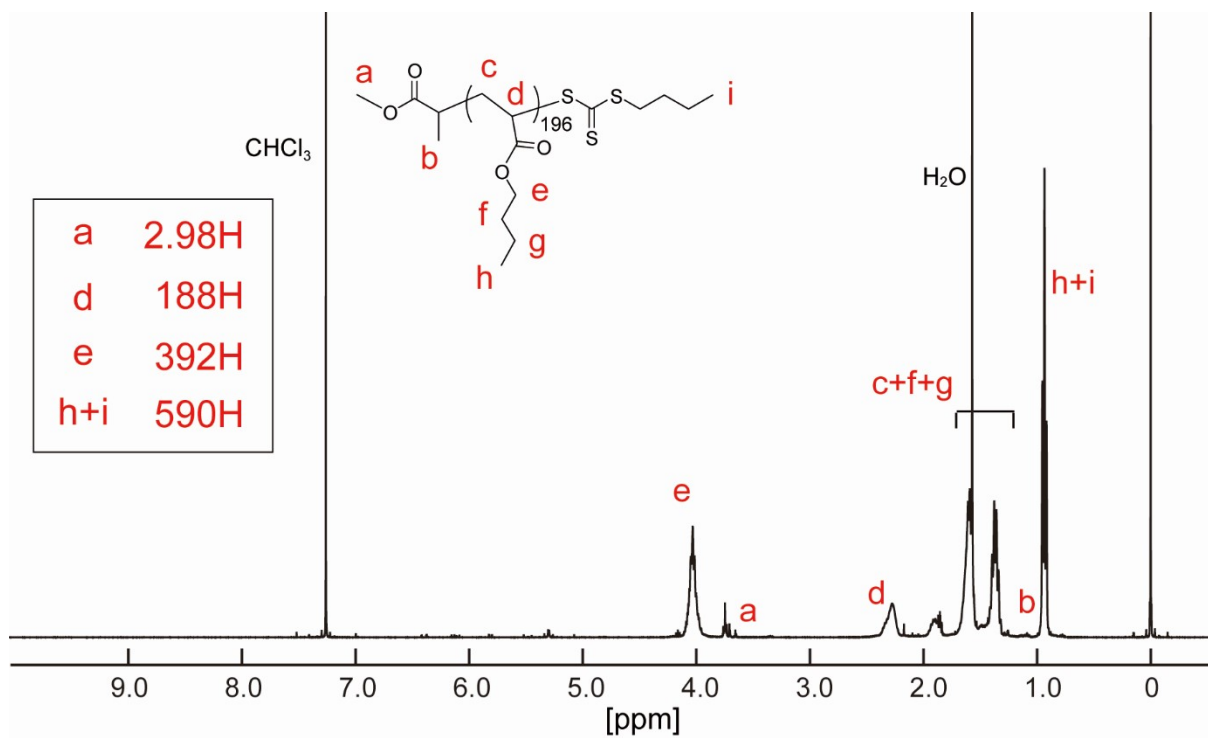
Figure S3. ^{1}H NMR spectrum of polyBA$_{196}$ (400 MHz, CDCl$_3$).

a   2.98H
d   188H
e   392H
h+i   590H
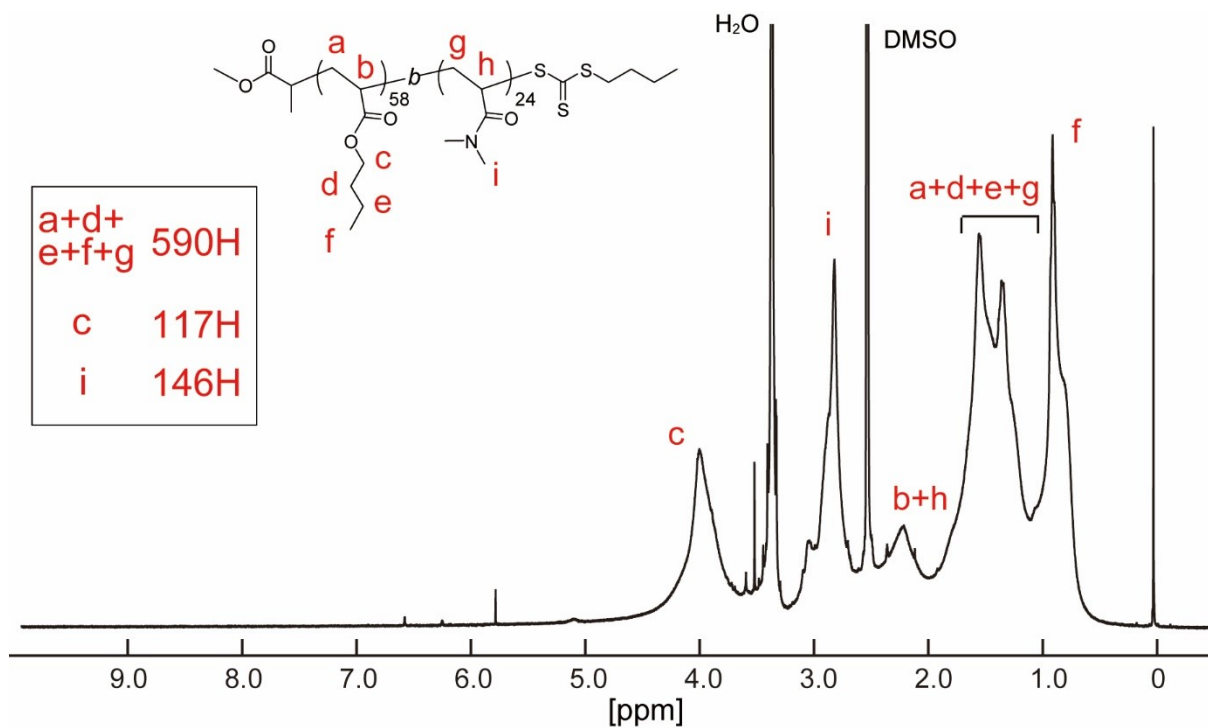
Figure S4. ^{1}H NMR spectrum of **B$_{58}$D$_{24}$** (400 MHz, DMSO-d_6).

a+d+e+f+g   590H
c   117H
i   146H

5
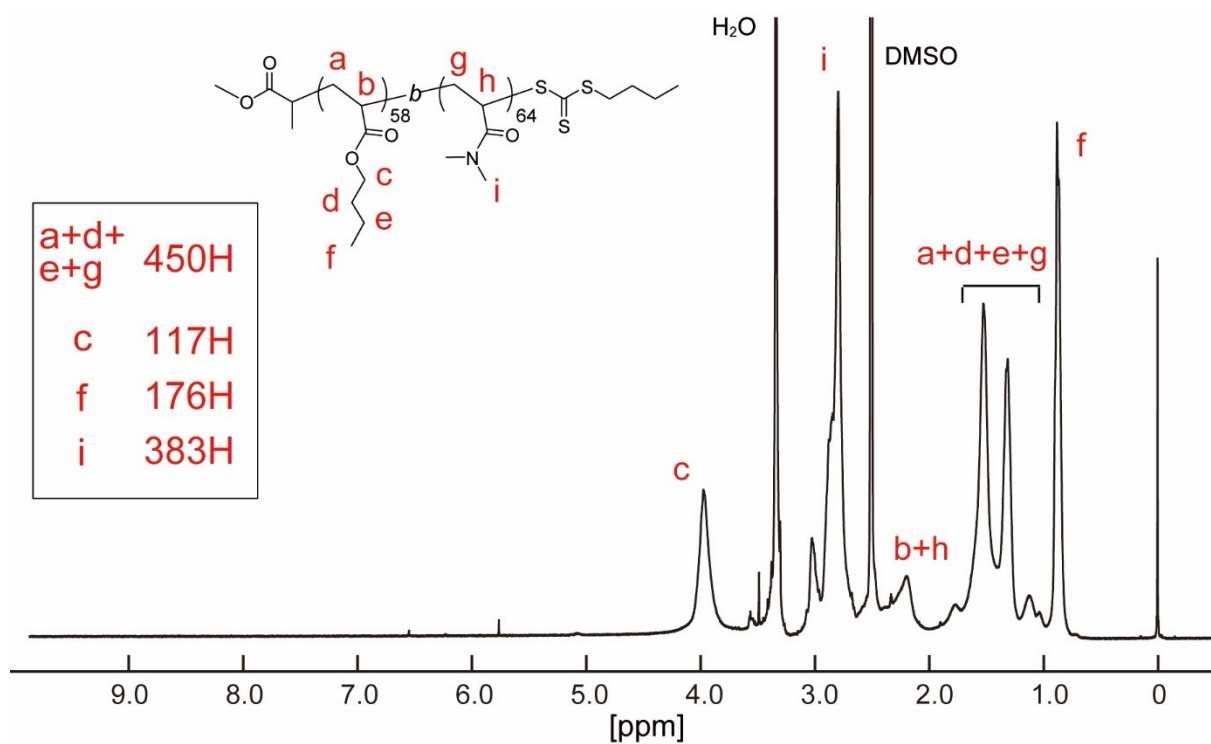
Figure S5. ^{1}H NMR spectrum of **B$_{58}$D$_{64}$** (400 MHz, DMSO-d_6).
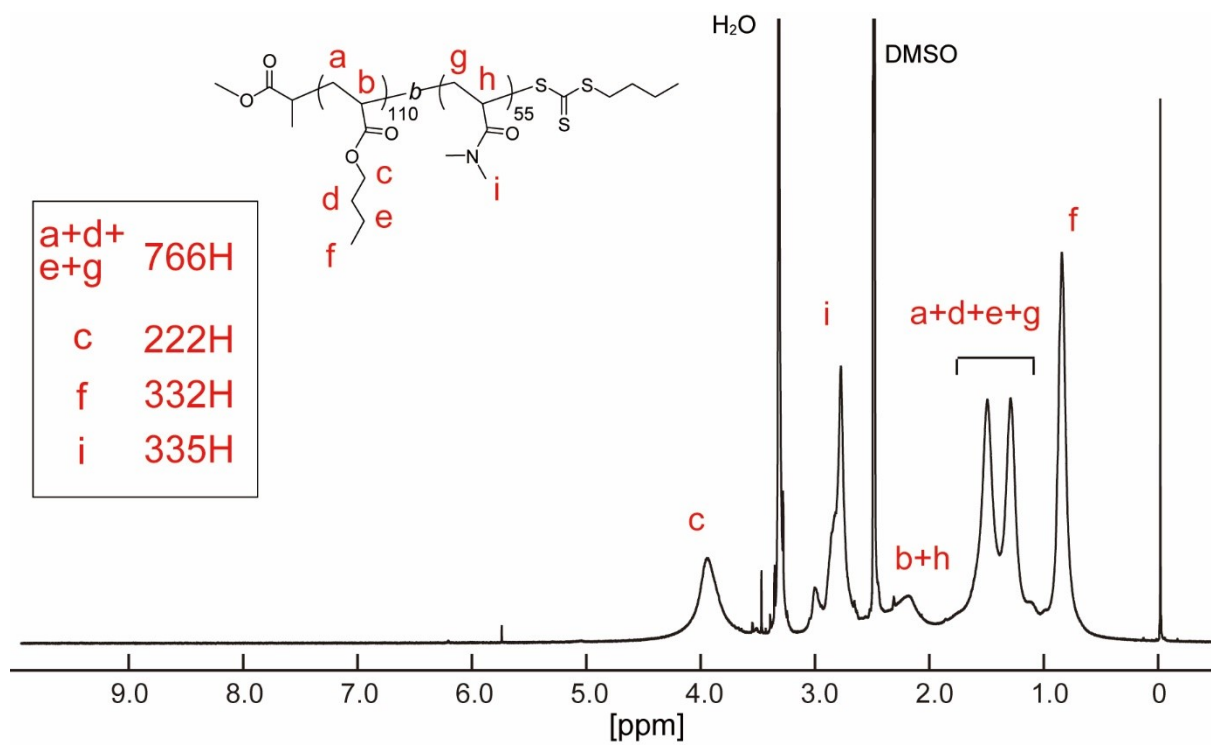
Figure S6. ^{1}H NMR spectrum of **B$_{110}$D$_{55}$** (400 MHz, DMSO-d_6).

Figure S7. ^{1}H NMR spectrum of **B$_{110}$D$_{110}$** (400 MHz, DMSO-d_6).

Figure S8. ^{1}H NMR spectrum of **B$_{196}$D$_{121}$** (400 MHz, DMSO-d_6).
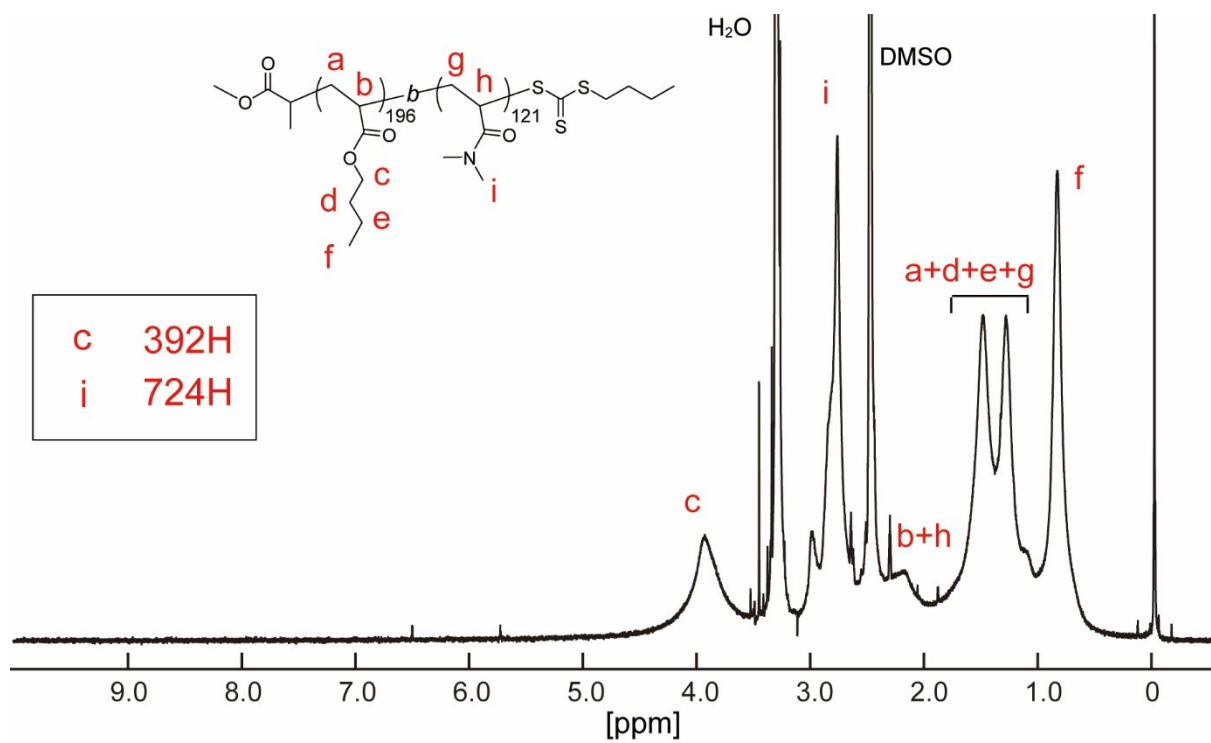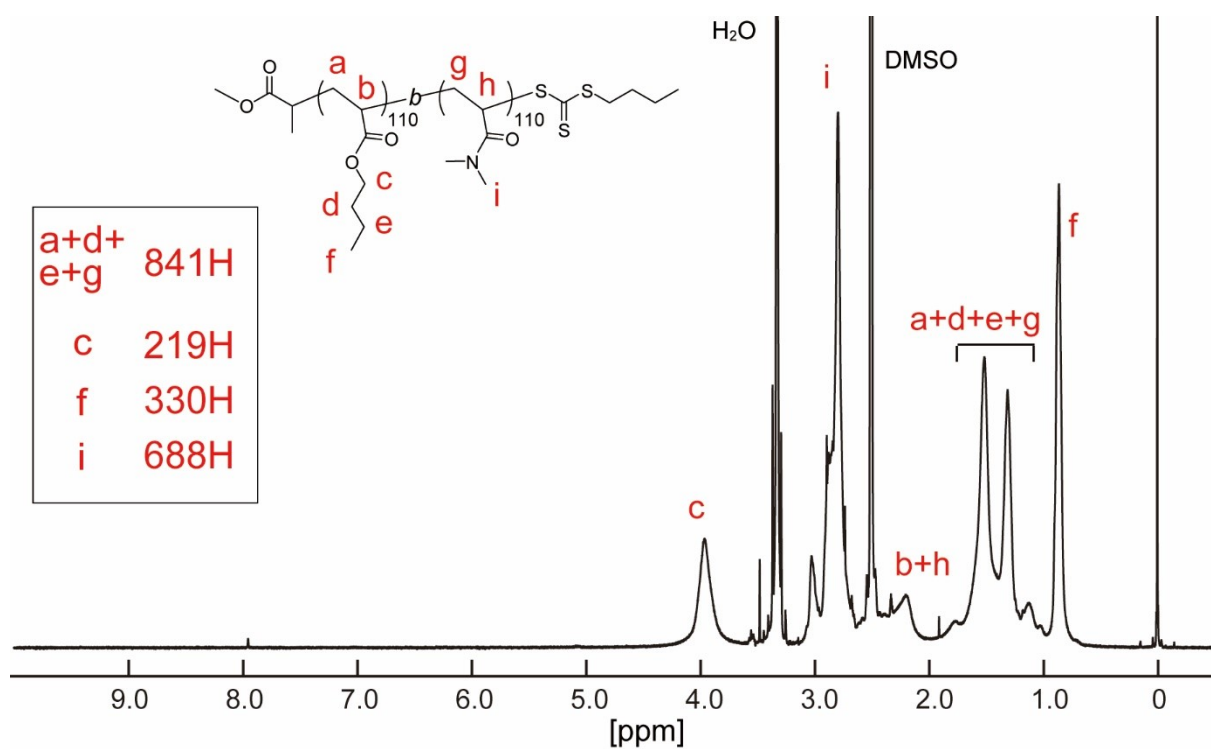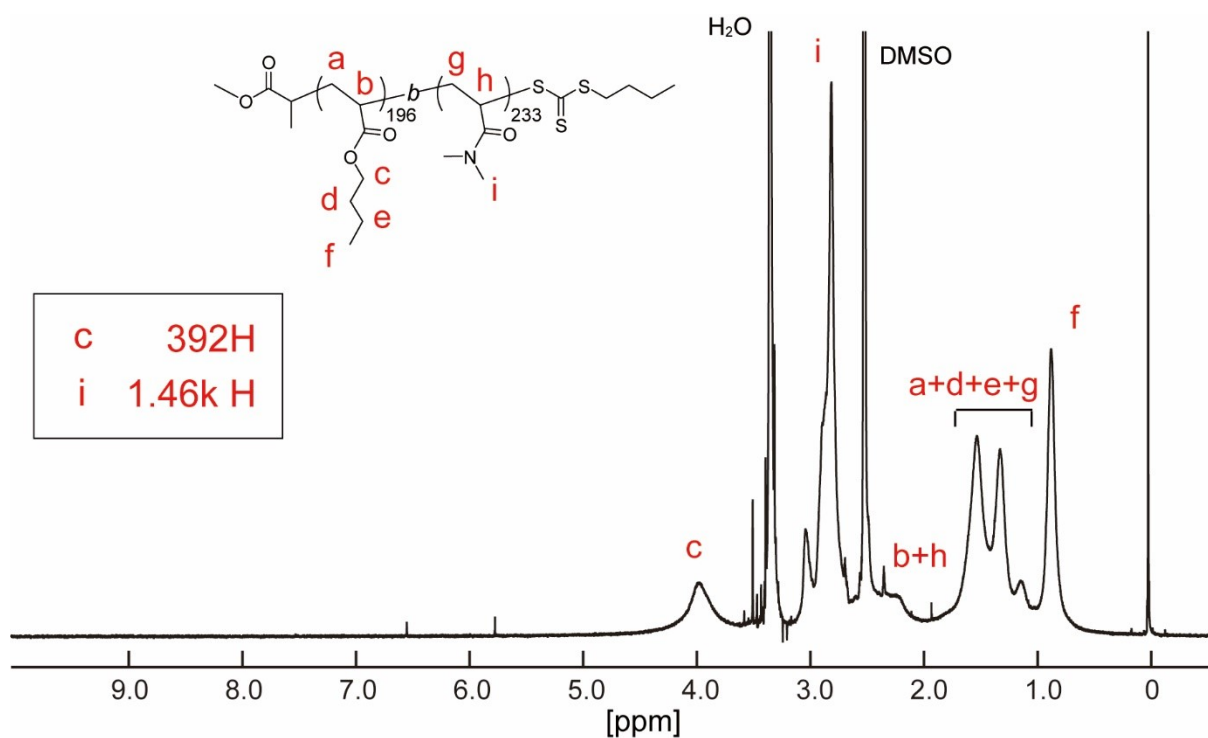
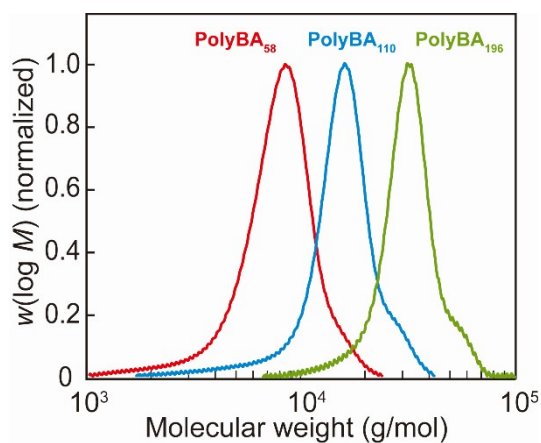Figure S9. ^{1}H NMR spectrum of $\mathbf{B_{196}D_{233}}$ (400 MHz, DMSO-d_6).

Figure S10. Molecular weight distributions of polyBA series. Red, blue, and green lines indicate polyBA$_{58}$, polyBA$_{110}$ and polyBA$_{196}$, respectively. The eluent was DMF with 10 mM LiBr.
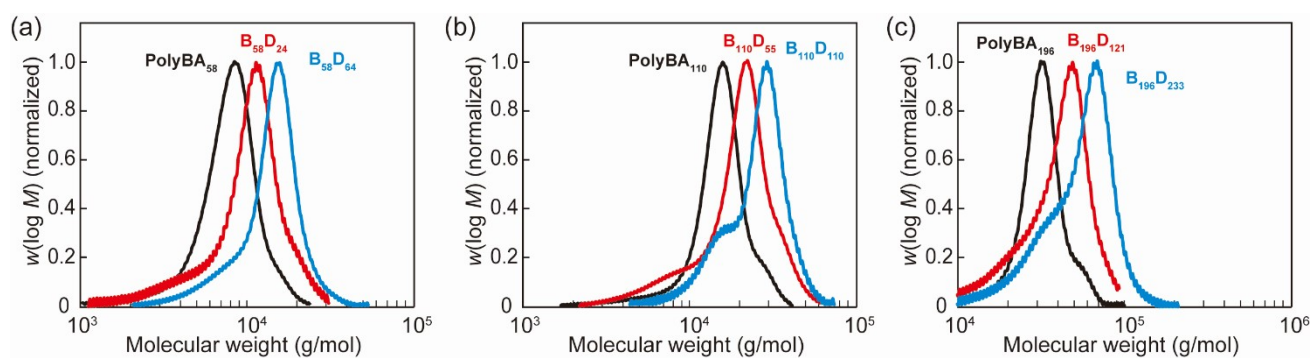
Figure S11. Molecular weight distributions of the amphiphilic diblock copolymers prepared with polyBA$_{58}$ (a), polyBA$_{110}$ (b), and polyBA$_{196}$ (c). Red and blue lines indicate the diblock copolymers, and black lines indicate polyBA polymers. The eluent was DMF with 10 mM LiBr.

3. Dynamic light scattering measurement

The synthesized diblock copolymers were dissolved in ethanol (10 g/L). The polymer solution (10 μL) was dropped into the PBS solution (1 mL) with stirring. The polymer solution in PBS (0.1 g/L) was analyzed by DLS without filtering.
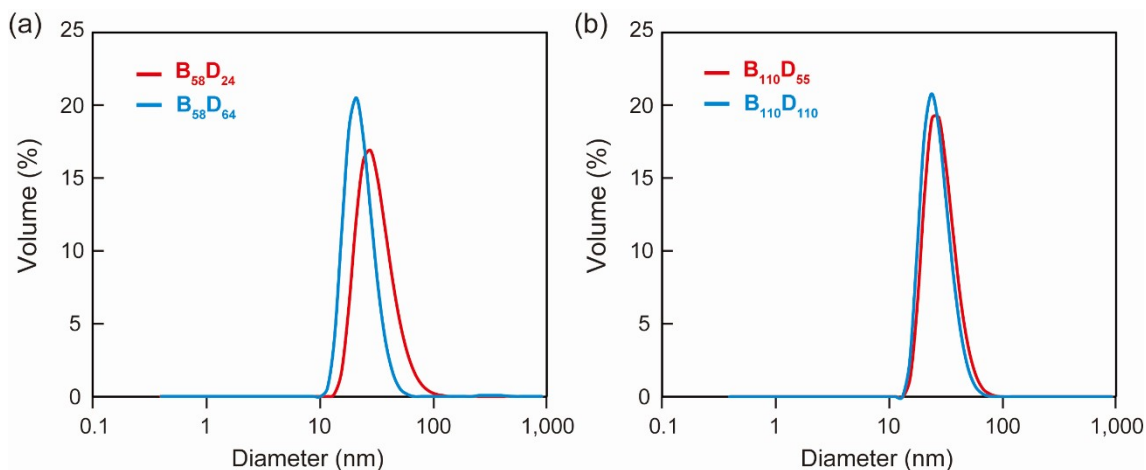

Figure S12. Dynamic light scattering distributions of the amphiphilic diblock copolymers prepared with polyBA$_{58}$ (a) and polyBA$_{110}$ (b) based on volume. The polymer concentration was 0.1 g/L in PBS. The temperature was 25 °C.

4. Membrane preparation with the solvent-assisted method

The polymer membrane was produced directly onto QCM silica sensors and monitored using a QCM-D device. First, the QCM sensors were rinsed with water and ethanol and placed in a UV ozone cleaner for 10 min to remove contaminants from the surface. The resulting sensors were immediately used for layer deposition. The amphiphilic copolymer was dissolved in ethanol (10 mg/L). The flow rate was kept at a constant value of 0.1 mL/min during the experiment or 0.05 mL/min during the protein adsorption. After PBS and ethanol injection in the QCM chamber and the baseline stabilization (steps 1 and 2 for Figure 2, S4, S5 and S6), the polymer solution was injected for 10 min at a constant flow of 0.1 mL/min (step 3) followed by buffer exchange with PBS for other 10 min (step 4). A solution of BSA dissolved in PBS (0.5 g/L) was injected in the QCM device for 15 min (step 5) followed by PBS rinsing for 10 minutes (step 6). BSA adsorption onto silicon dioxide led to a frequency shift of −30 Hz (Figure S7).
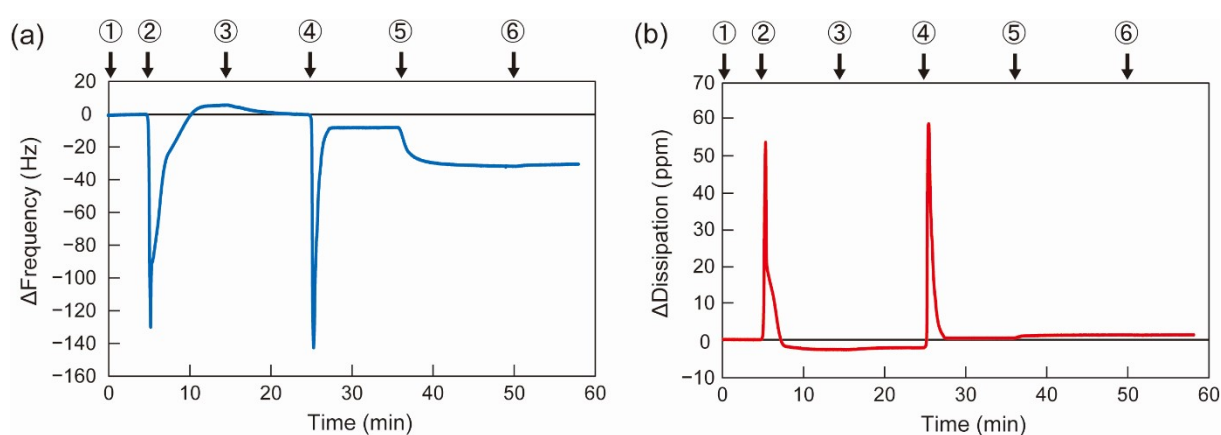
Figure S13. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with $B_{58}D_{24}$: PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), and PBS (6).
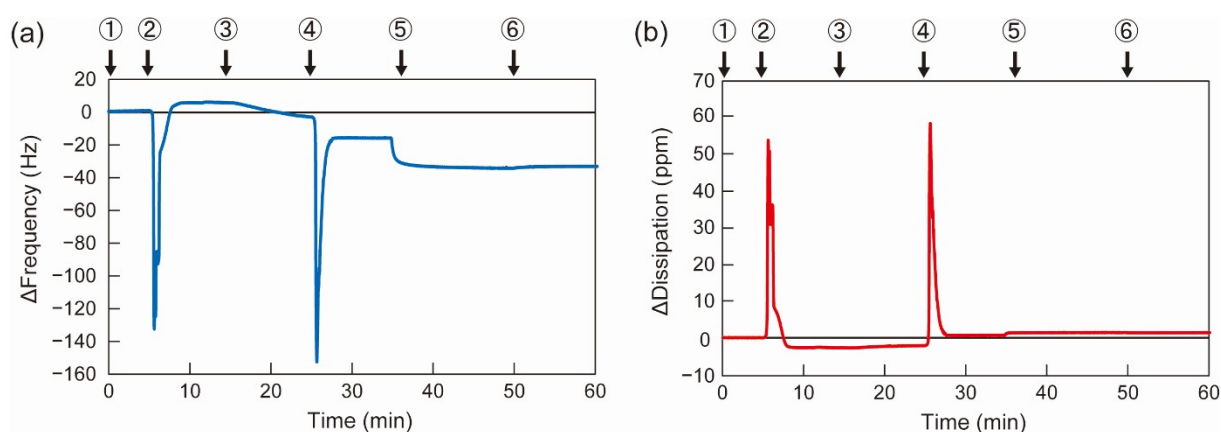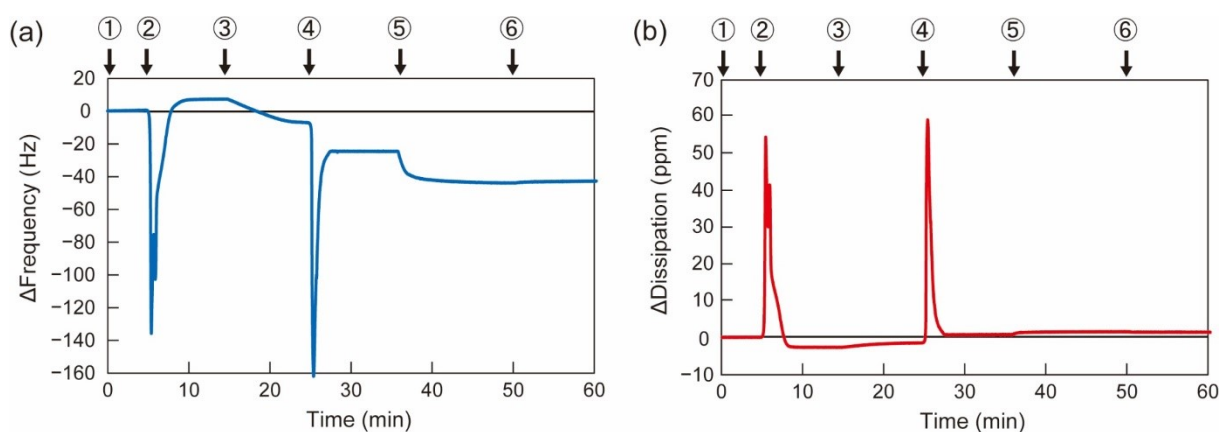
Figure S14. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with $B_{58}D_{64}$: PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), and PBS (6).

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Figure S15. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with $B_{110}D_{55}$: PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), and PBS (6).
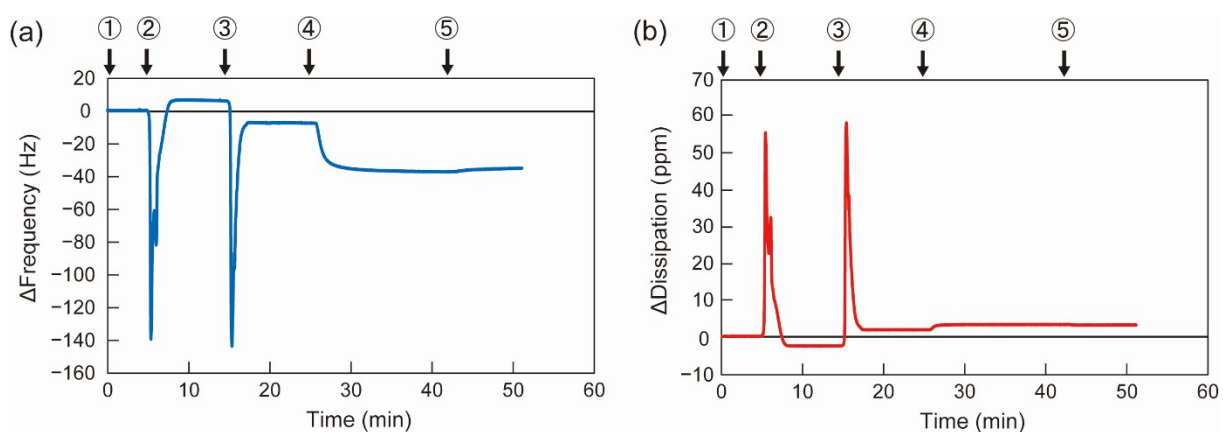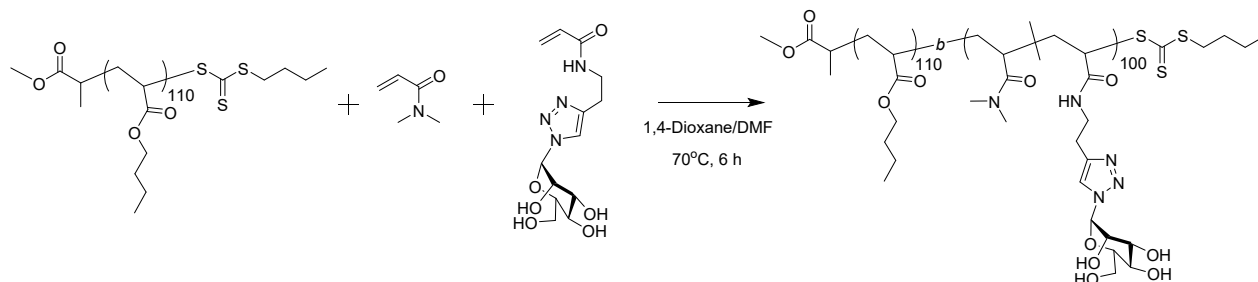

Figure S16. QCM-D frequency (a) and dissipation (b) profiles for BSA adsorption: PBS (1), ethanol (2), PSB (3), BSA solution in PBS (4), and PBS (5).

5. Synthesis of the amphiphilic glycopolymers

5.1. Synthesis of the amphiphilic glycopolymers with random sequence (**RG**)

DMA (69 mg, 700 μmol), ManAAm (29 mg, 88 μmol), polyBA$_{110}$ (100 mg, 7.1 μmol) and AIBN (0.14 mg, 0.87 μmol) were dissolved in a mixture of 1,4-dioxane (320 μL), DMF (50 μL), and water (17 μL). The solution was prepared in a glass tube and degassed by freeze−thaw cycles (three times). The glass tube was sealed and put in an oil bath. The reaction proceeded at 70 °C for 6 h. The reaction was stopped by exposing the solution to air. The monomer conversion was determined by ^{1}H NMR (CDCl$_3$). The polymer solutions were purified by dialysis against Milli-Q water (MWCO = 3.5k). The objective was obtained by freeze-drying.

5.2. Synthesis of the amphiphilic glycopolymers with block sequence (**BG**)
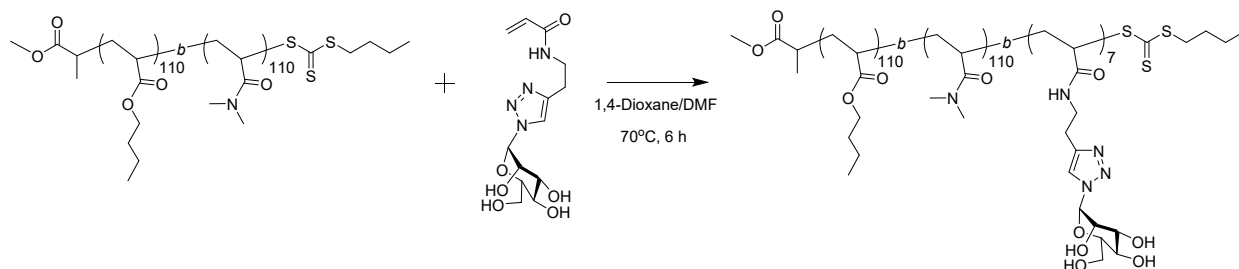
ManAAm (4 mg, 12 μmol), **B$_{110}$D$_{110}$** (50 mg, 2 μmol) and AIBN (0.04 mg, 0.24 μmol) were dissolved in a mixture of 1,4-dioxane (240 μL), DMF (40 μL), and water (20 μL). The solution was prepared in a glass tube and degassed by freeze−thaw cycles (three times). The glass tube was sealed and put in an oil bath. The reaction proceeded at 70 °C for 6 h. The reaction was stopped by exposing the solution to air. The monomer conversion was determined by ^{1}H NMR (CDCl$_3$). The polymer solutions were purified by dialysis against Milli-Q water (MWCO = 3.5k). The objective was obtained by freeze-drying.
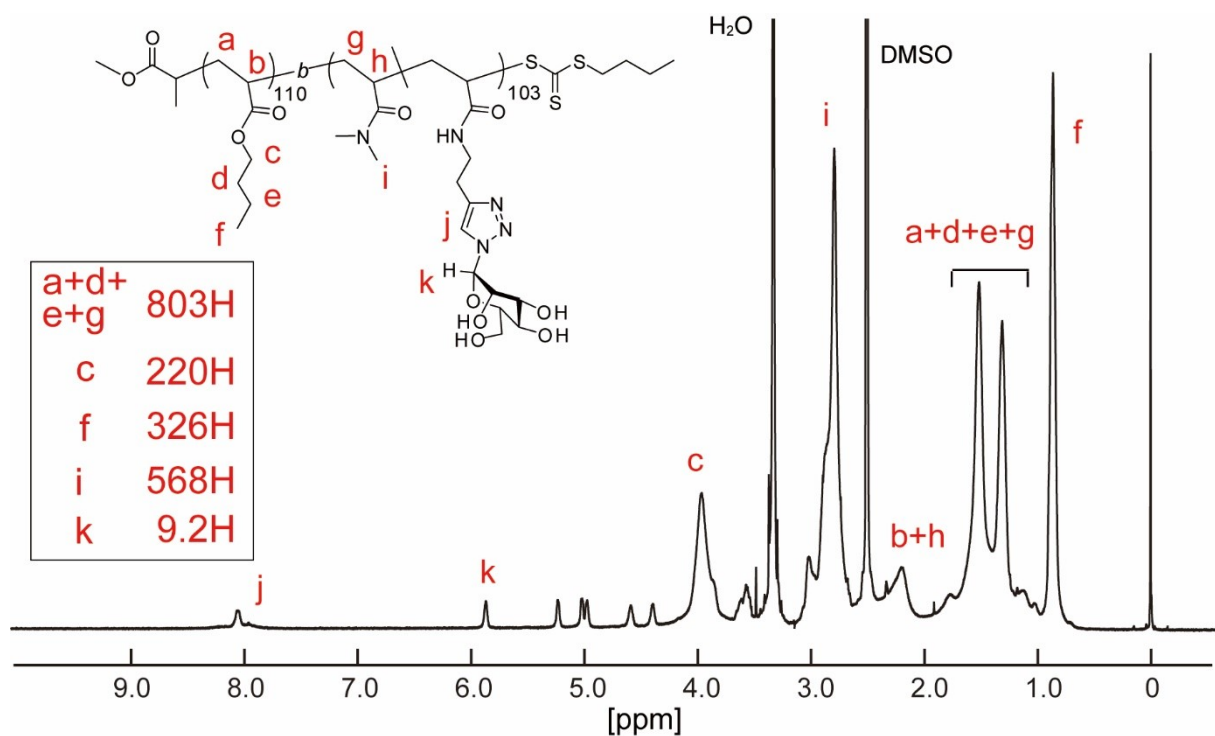
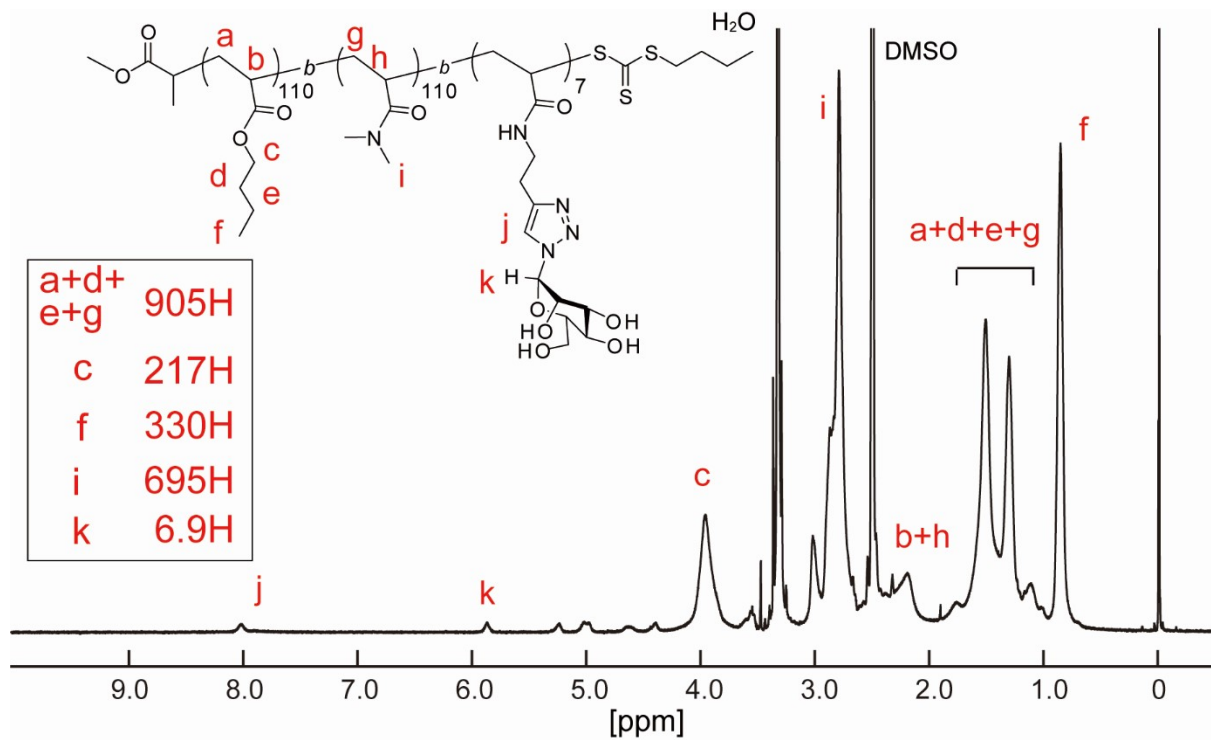Figure S17. ^{1}H NMR spectrum of **RG** (400 MHz, DMSO-d_6).

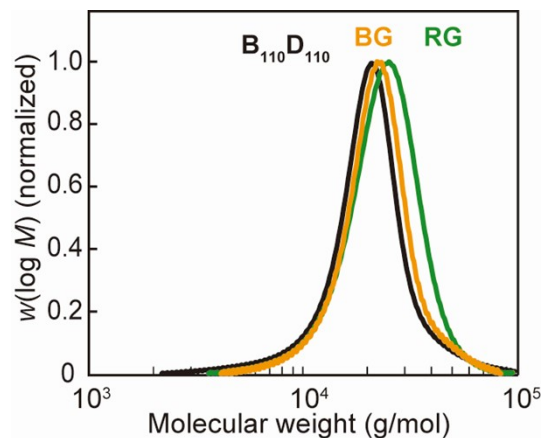Figure S18. ^{1}H NMR spectrum of **BG** (400 MHz, DMSO-d_6).

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Figure S19. Molecular weight distributions of the amphiphilic copolymers. Black, green, and yellow lines indicate $B_{110}D_{110}$, RG and BG, respectively. The eluent was DMF with 10 mM LiBr.
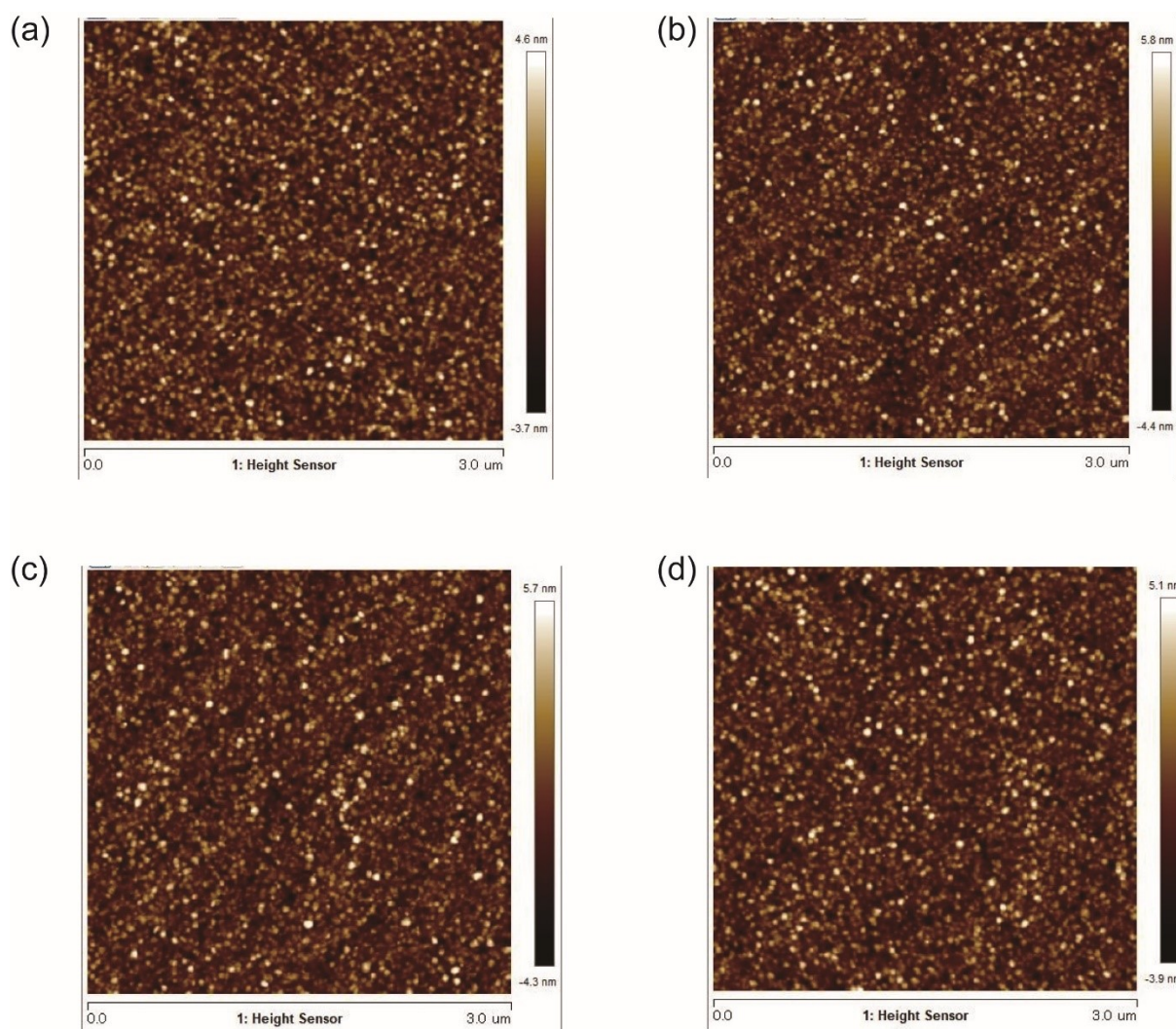

Figure S20. AFM phase images of different polymer membranes: (a) bare glass surface, (b) $B_{110}D_{110}$,

(c) **RG**: $B_{110}D_{110}$ = 50: 50 wt%, and (d) **BG**: $B_{110}D_{110}$ = 50: 50 wt%.

6. Evaluation of the interaction of glycopolymer interface

Either of the glycopolymers (**RG** or **BG**) and the amphiphilic copolymer ($\mathbf{B_{110}D_{110}}$) were mixed at different mass ratios and dissolved in ethanol (10 mg/L). The flow rate was kept at a constant value of 0.1 mL/min during the experiment or 0.05 mL/min during the protein adsorption. After PBS and ethanol injection in the QCM chamber and the baseline stabilization (steps 1 and 2 for Figure 4a-b, S10, S11 and S12), the polymer solution was injected for 10 min at a constant flow of 0.1 mL/min (step 3) followed by buffer exchange with PBS for other 10 min (step 4). A solution of BSA dissolved in PBS (0.5 g/L) was injected in the QCM device for 15 min (step 5) followed by PBS(+) rinsing for 5 minutes (step 6). A solution of ConA dissolved in PBS(+) with the concentration of 0.01 g/L (step 7) and 0.1 g/L (step 8) was injected in the QCM device for 15 min each followed by PBS(+) rinsing for 10 minutes (step 9). For the evaluation using PNA, PBS(+) was replaced with to PBS(-).

For preparation of the glycopolymer membrane by spin-coating, polymer solution ($\mathbf{B_{110}D_{110}}$: **RG** = 50: 50 wt%) in ethanol (10 mg/L) was spin-coated onto the SiO_2 sensor of QCM-D with a spin coater (model 1H-DX2, Mikasa Co., Ltd, Tokyo, Japan) at 2000 rpm for 30 s and then dried at room temperature. The sensor chip was set in the module, and signal was stabilized with flowing PBS solution. The same method was applied after step 5 of the above method.
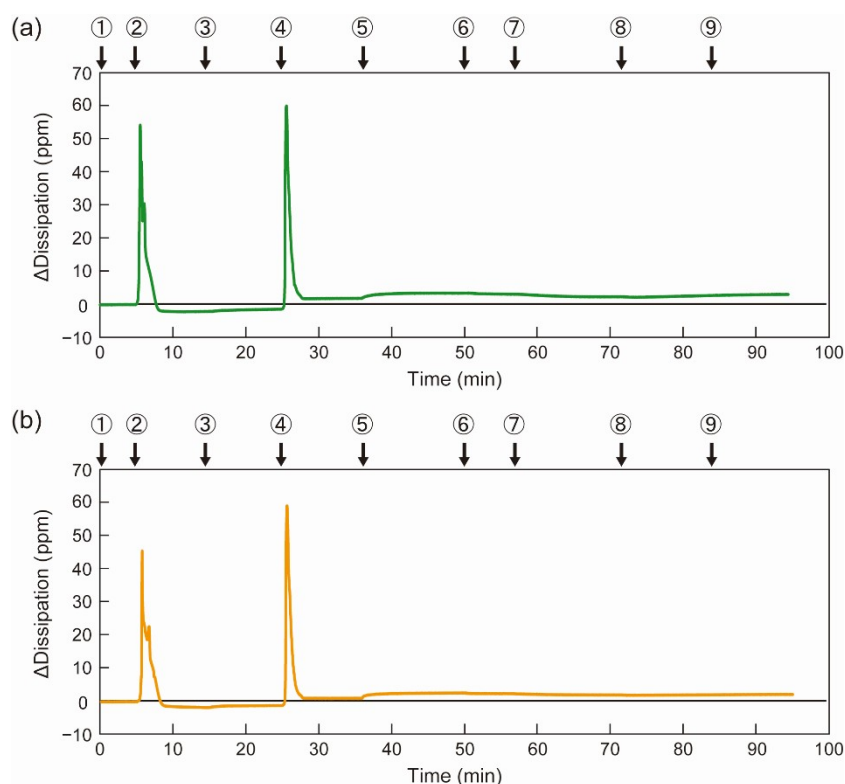

Figure S21. QCM-D dissipation profiles for polymer membrane formation with $\mathbf{B_{110}D_{110}}$: **RG** = 50: 50 (w/w) (a) and with $\mathbf{B_{110}D_{110}}$: **RG** = 50: 50 (b). In the profiles, PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), PBS(+) (6), ConA solution in PBS(+) (C = 0.01 g/L) (7), ConA solution in PBS(+) (C = 0.1 g/L) (8), and PBS (+) (9).

Table S3. Frequency changes of the glycopolymer membranes with 50 wt% of **RG** or **BG** in ConA adsorption.

ConA	$B_{110}D_{110}$: **RG** = 50: 50 wt%				$B_{110}D_{110}$: **BG** = 50: 50 wt%			
	n = 1	n = 2	n = 3	Average	n = 1	n = 2	n = 3	Average
0.01 g/L	13.9	20.0	21.0	18 ± 3.2	6	8.0	7.4	7.1 ± 0.8
0.1 g/L	36.9	39.0	33.0	36 ± 2.5	14	18.0	20.0	17 ± 2.5

Table S4. Frequency changes of the glycopolymer membranes with 50 wt% of **RG** or **BG** in PNA adsorption.

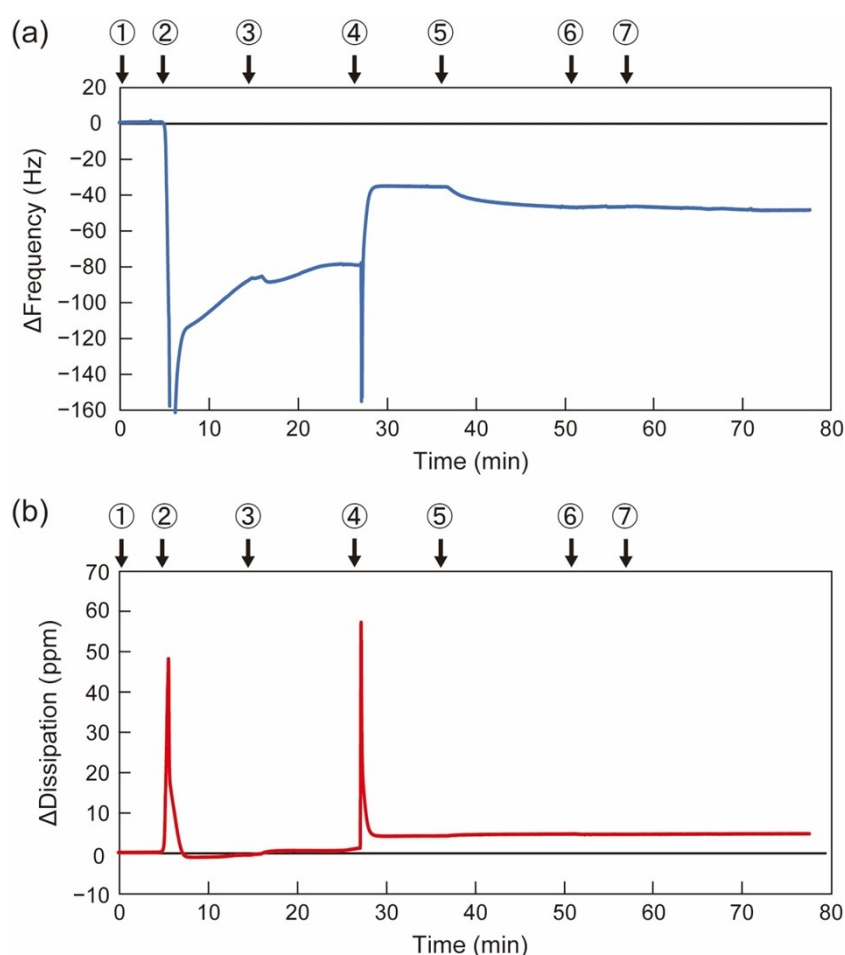| PNA	$B_{110}D_{110}$: **RG** = 50: 50 wt%	$B_{110}D_{110}$: **BG** = 50: 50 wt%
0.1 g/L	1.7	2.4

Figure S22. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with $B_{110}D_{110}$: **RG** = 50: 50 (w/w): PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), PBS (6), PNA solution in PBS (C = 0.1 g/L) (7).
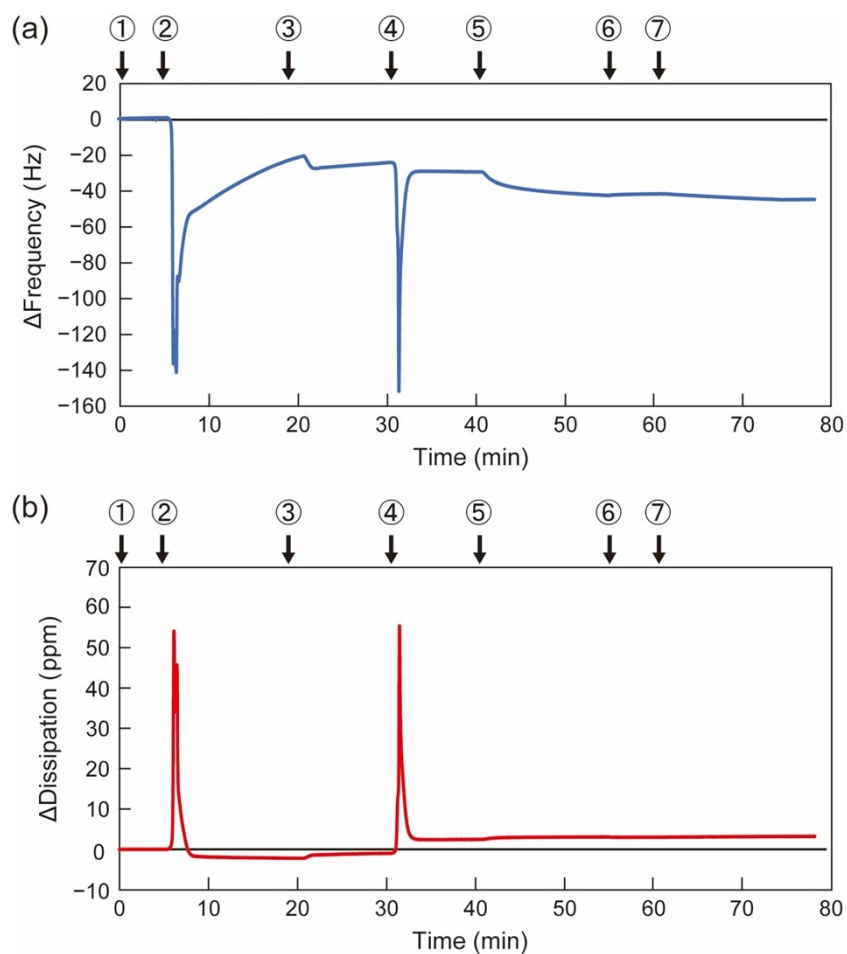
Figure S23. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with **B$_{110}$D$_{110}$**: **BG** = 50: 50 (w/w): PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), PBS (6), PNA solution in PBS (C = 0.1 g/L) (7).
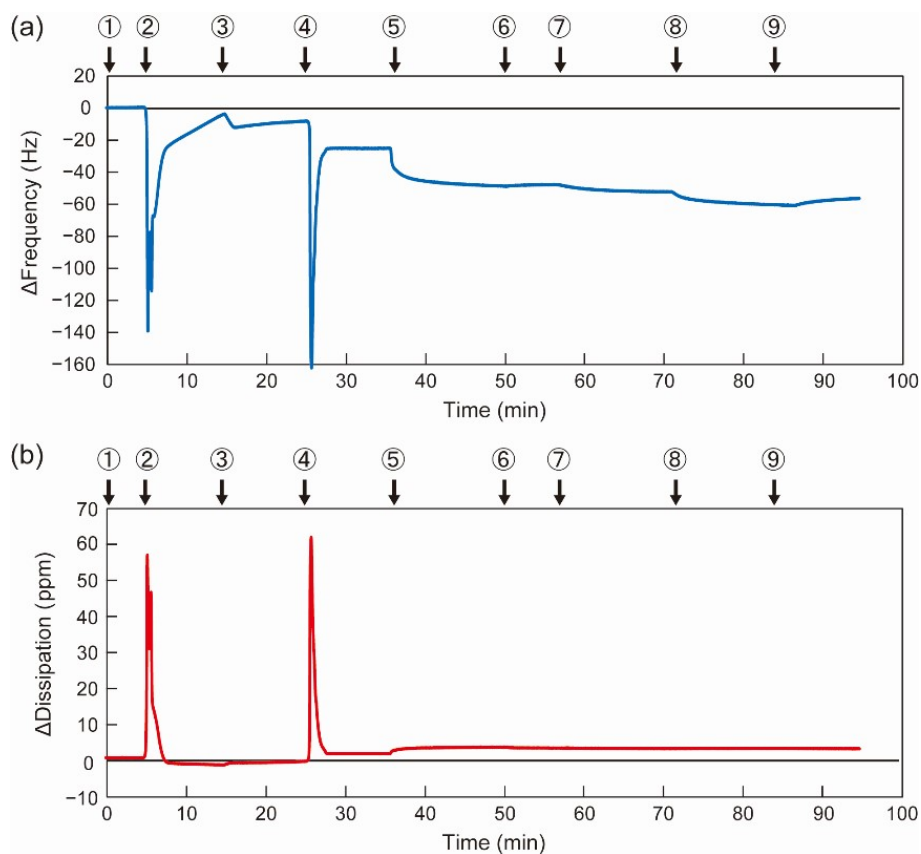
Figure S24. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with **B$_{110}$D$_{110}$**: **RG** = 10: 90 (w/w): PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), PBS(+) (6), ConA solution in PBS(+) (C = 0.01 g/L) (7), ConA solution in PBS(+) (C = 0.1 g/L) (8), and PBS (+) (9).
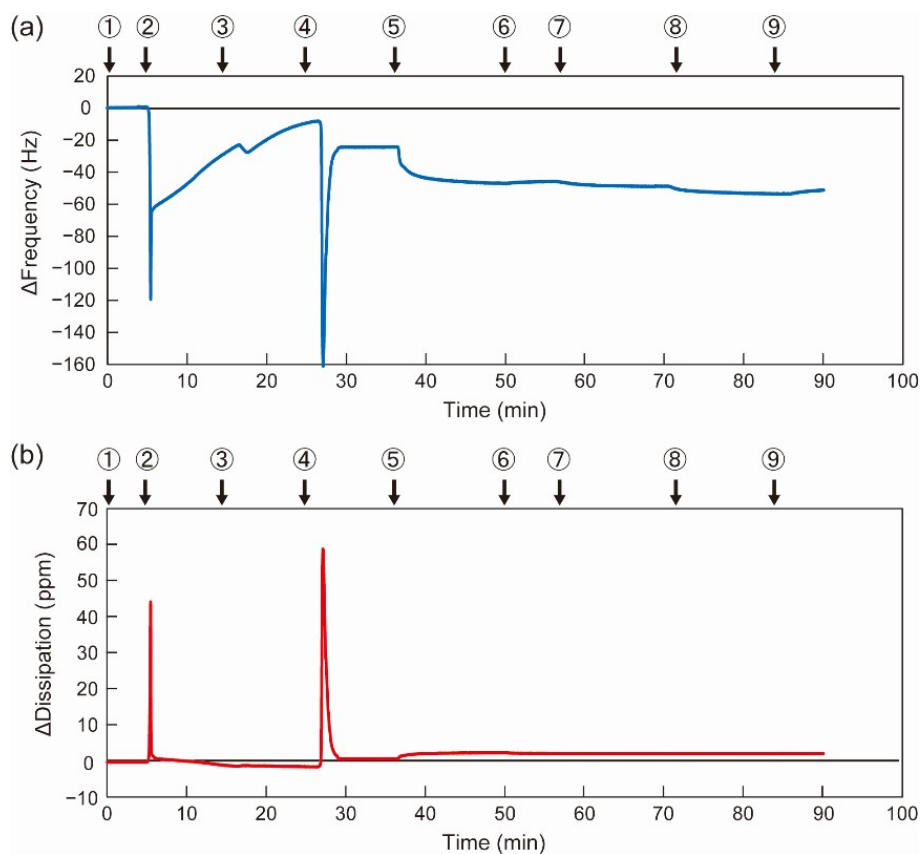
Figure S25. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with **B₁₁₀D₁₁₀**: **BG** = 10: 90 (w/w): PBS (1), ethanol (2), polymer solution in ethanol (3), PBS (4), BSA solution in PBS (5), PBS(+) (6), ConA solution in PBS(+) (C = 0.01 g/L) (7), ConA solution in PBS(+) (C = 0.1 g/L) (8), and PBS (+) (9).
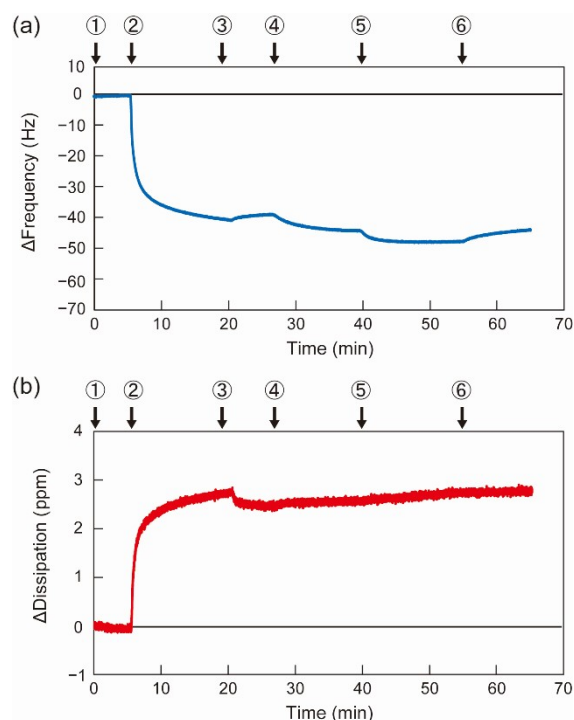
Figure S26. QCM-D frequency (a) and dissipation (b) profiles for polymer membrane formation with **B₁₁₀D₁₁₀**: **BG** = 50: 50 (w/w) by spin-coating: PBS (1), BSA solution in PBS (2), PBS(+) (3), ConA solution in PBS(+) (C = 0.01 g/L) (4), ConA solution in PBS(+) (C = 0.1 g/L) (5), and PBS (+) (6).
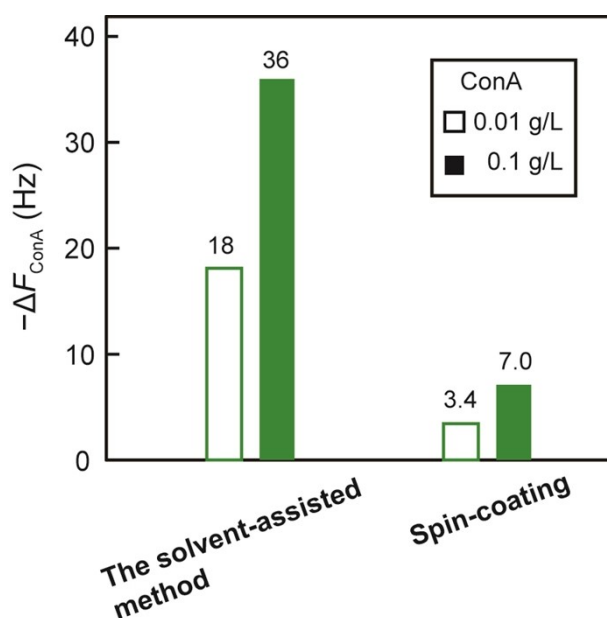

Figure S27. The frequency change in ConA adsorption for the glycopolymer membranes in different preparation methods. The composition was **B₁₁₀D₁₁₀**: **RG** = 50: 50 (w/w). The polymer concentration was 10 mg/L in ethanol.

Reference:

(1) M. Nagao, Y. Fujiwara, T. Matsubara, Y. Hoshino, T. Sato and Y. Miura, *Biomacromolecules* **2017**, *18*, 4385–4392.

(2) M. Nagao, M. Kichize, Y. Hoshino and Y. Miura, *Biomacromolecules*, **2021**, *22*, 3119–3127.